(19)

Europäisches Patentamt

European Patent Office

Office européen des brevets

(11)   **EP 0 985 146 B1**

(12)   **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention
of the grant of the patent:
02.03.2005 Bulletin 2005/09

(51) Int Cl.7: **G01N 30/60, G01N 27/447,
G01N 30/48**

(21) Application number: **98922321.9**

(86) International application number:
PCT/US1998/009960

(22) Date of filing: **18.05.1998**

(87) International publication number:
WO 1998/054568 (03.12.1998 Gazette 1998/48)

(54) **IMPROVED SEPARATION COLUMNS AND METHODS FOR MANUFACTURING THE IMPROVED SEPARATION COLUMNS**

VERBESSERTE TRENNSÄULEN UND VERFAHREN ZUR HERSTELLUNG DER VERBESSERTEN TRENNSÄULEN

COLONNES DE SEPARATION AMELIOREES ET LEURS PROCEDES DE FABRICATION

(84) Designated Contracting States:
DE FR GB

(30) Priority: **27.05.1997 US 863543**

(43) Date of publication of application:
15.03.2000 Bulletin 2000/11

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(56) References cited:
**WO-A-96/42012   US-A- 5 116 495
US-A- 5 427 663   US-A- 5 486 335**

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Description

Statement Regarding Federally Sponsored Research

The Field of the Invention

[0002]   The invention relates to separation apparatus and more specifically to separation columns and methods for manufacturing separation columns for use in separation processes.

Background

[0003]   Separation based analytical methods, including chromatography, electrophoresis and electrochromatography are useful in determining individual samples in complex mixtures. In chromatography, a sample to be analyzed is introduced into a separation column, which contains a mobile phase and a stationary phase. Components of the sample separate as the sample passes through the column due to differences in interaction of the different components with the stationary phase.

[0004]   Electrophoresis is a separation technique that is based on the mobility of ions in an electric field. In capillary electrophoresis, a sample is placed in a capillary tube, which contains an electrophoretic medium. Upon application of an electric field across the capillary, components of the sample migrate at different rates towards the oppositely charged ends of the tube based on their relative electrophoretic mobilities in the medium. Electrochromatography is a combination of chromatography and electrophoresis, in which the mobile phase is transported through the separation system by electroosmotic flow (EOF).

[0005]   Separation of samples in complex mixtures based on analytical systems that are capable of executing large numbers of separations would be useful. In particular, separation technologies that process multiple samples quickly and multi-dimensional separations for each sample are desired. However, existing separation technologies do not generally have these features. Liquid chromatography systems do not readily adapt to parallel processing because adding multiple precision pumps and valves, necessary to deliver multiple samples, is both impractical and expensive. Multi-dimensional chromatography separations are feasible by transferring components from a first separation column to a second separation column with rotary valves. However, such processes can be very slow. Parallel processing for capillary electrophoresis has been achieved using microfabrication, which allows multiple

channels to be placed on a single chip. However, a limitation is that no methods are available to introduce a large number of samples into the channels and to rapidly clean the sample metering channels between separations.

[0006]   US-A-5 116 495 describes a capillary chromatographic device in which the capillary column is formed between a cover layer and a substrate. The liquid distribution or splitting system of this prior art essentially only comprises an inlet opening and an outlet opening both communicating with a larger space, thereby allowing liquid to spread into the capillary column or capillary columns.

[0007]   WO 96/42012 describes a nanofabricated separation matrix for analysis of biopolymers and methods of making and using the same. The separation matrix has a solid support on which are disposed a plurality of lanes respectively containing a plurality of posts which form an obstacle course for the separation of the biopolymers. The spacing between the posts is defined so as to permit passage of the biopolymer to be separated, yet small enough to provide an obstacle to passage which will result in separation.

[0008]   US-A-5 427 663 describes a microlithographic array for macromolecule and cell fractionation which in a similar manner has an array of obstacles formed on a substrate and positioned within a receptacle.

[0009]   It is the object of the present invention to provide a separation column which can be manufactured in a simple manner and which is improved with respect to the flow of the samples through the separation column. The present invention also aims at providing a method for manufacturing such separation column, a separation apparatus using such separation column and method for separating components of a sample by using such separation column.

Summary of the Invention

[0010]   In one aspect, the invention relates to a separation column as defined in claim 1 to be used in a separation process. The separation column includes multiple collocated monolith support structures and interconnected channels defined by the support structures. The collocated monolith support structures are arrayed in two dimensions to define channels that periodically split and merge. In one embodiment, the support structures are in communication with each other at the first end of each support structures and a cover plate is in communication with the support structures at the second end of each structure.

[0011]   In another aspect, the invention relates to a method as defined in claim 22 for manufacturing a separation column, which is to be used in a separation process. According to the method, a substrate is patterned to designate the areas of the substrate to be etched. The patterned substrate is etched to create multiple collocated monolith support structures arrayed in two dimen-

sions and interconnected channels defined by the support structures. In one embodiment, a cover plate is attached on a surface of the created support structures to enclose the separation column. In another embodiment, the substrate is etched to create the support structures and the channels that are substantially uniform in shape and size. In yet another embodiment, the substrate is etched to create interconnected channels, in which each channel has an aspect ratio of from about 5 to about 100. The aspect ratio as used herein is the ratio of the depth to the width of a channel between adjacent monolith structures, where the depth is a dimension perpendicular to the surface of the substrate and the width is a dimension parallel to the surface of the substrate and perpendicular to the flow direction in the channel.

[0012] The separation column includes an inlet, a separation region and an outlet. The separation region is in communication with the inlet and comprises a plurality of collocated monolith support structures that are arranged in two dimensions. The support structures define a plurality of interconnected channels that sequentially split and merge. The outlet is in communication with the separation region.

[0013] In yet another aspect, the invention relates to a separation apparatus as defined in claim 26. The separation apparatus includes a separation column, a plurality of reservoirs for mobile phases or buffers and a sample reservoir. The separation column has multiple collocated support structures arrayed in two dimensions and interconnected channels defined by the support structures. The reservoirs are in communication with the separation column. The sample reservoir is in communication with the separation column. In one embodiment, the separation apparatus also includes a pump for pumping a mobile phase from a reservoir through the separation column. In another embodiment, the separation apparatus also includes an electrophoresis apparatus..

[0014] In still another aspect, the invention relates to a method as defined in claim 31 for separating components of a sample. According to the method, a medium solution is introduced into a separation column, which includes multiple collocated monolith support structures and interconnected channels defined by the support structures. A sample to be analyzed is also introduced into the separation column. The solution and the sample pass through the separation column in multiple streams and the multiple streams periodically intercouple. Components of the sample are separated as the sample passes through the column. The components may be separated by electrophoretic mobility, electroosmotic flow (EOF), EOF and partitioning with a stationary phase, micellar electrokinetic chromatography, or a combination of these.

Brief Description of the Drawings

[0015] These and other advantages of the invention may be more clearly understood with reference to the specification and the drawings, in which:

Figure 1A shows a perspective view of an embodiment of a separation column with collocated monolith support structures constructed in accordance with the invention.

Figure 1B shows a detailed planar view of a portion of the embodiment of the separation column of Figure 1A.

Figure 1C shows a cross-sectional view of the section of the embodiment of the separation column of Figure I A cut through the line 1C'-1C".

Figure 1D shows a cross-sectional view of a section of an embodiment of the separation column of Figure 1A.

Figures 2A, 2B, 2C, 2D, 2E and 2F depict a plan view of several embodiments of the monolith support structures useful in the embodiment of the invention shown in Figure 1A.

Figures 3A, 3B, 3C and 3D depict a plan view of several additional embodiments of the monolith support structures useful in the embodiment of the invention shown in Figure 1A.

Figures 4A and 4B are highly schematic diagrams of the diagonal field line effect in various embodiments of the present invention.

Figures 5A, 5B and 5C show a plan view of embodiments of the support structures for eliminating wall-effects near the walls of a separation column.

Figure 6 shows a rendition of a plan view of an embodiment of a collocated monolith distributor of a second separation column interfaced with a collocated monolith collector of a first separation column.

Figure 7 shows an electropherogram of a separation performed by an embodiment of a separation apparatus.

Figure 8 shows an electropherogram of a separation performed by an embodiment of a separation apparatus.

Description of the Preferred Embodiment

[0016] Referring to Figures 1A, 1B 1C and 1D a separation column 10, constructed in accordance with the invention, includes a number of collocated monolith support structures 14 defining a series of interconnected mi-

crochannels 12. The term "collocated" refers to a side by side placement. The term "monolith" refers to a single structure, including a structure that forms a single piece by attachment. The collocated monolith support structures 14 arrayed in two dimensions and define channels 12 that periodically merge and split. The collocated monolith support structures 14 are fabricated on a substrate 11, and hence are attached to one another at a first end 7 by the substrate 11. However, the remainder of each monolith structure 14 is physically separated from each other forming interconnected channels 12. In the embodiment of Figure 1C, a cover plate 13 is disposed over and bonded to the second end 9 of the collocated monolith support structures 14 enclosing the separation column 10. In the embodiment of Figure 1D, a second group of monolith support structures 2 being a mirror image of a first group of monolith support structures 14 is disposed over the first group of monolith support structures 14 such that the second end 9 of a monolith structure 14 of the first group joins the second end 4 of a monolith structure 2 of the second group, thereby forming channels 6 that are twice as deep. The dimensions of the collocated monolith structures 14 are typically about less than 100 μm in height and 1000 μm² in cross-sectional area 18 for chromatography applications. The height of the monolith structures 14, however, may be much longer, for example 500 μm, in electrophoresis applications. In this context, height refers to the distance from a first end 7 to a second end 9 of a monolith structure 14, perpendicular to the surface of the substrate 11. Since, the present height of a monolith structure 14 is limited by existing etching technologies, the height is expected to increase with advance in such technologies. Cross-sectional area 18 refers to the area of a monolith structure 14 measured parallel to the plane of the substrate 11. The distance between any two adjacent monolith structures 22 is approximately equal and typically does not exceed about 10 μm at any point in chromatography applications. In electrophoresis applications, the distance may be much wider, for example 100 μm.

[0017] In the embodiment of Figures 1A, 1B, 1C and 1D the monolith structures 14 are substantially identical in size and shape in the separation column 10 and the channel walls 24 are as nearly vertical as possible, such that width of the channel 22 along an entire channel is approximately constant. With capillary electrophoresis, channels 12 do not necessarily have to be vertical. However, with pressure driven, open channel liquid chromatography, widths 22 along an entire channel should be constant, because a flow rate in parallel channels (i.e., at the same position along the column length) in a pressure driven system is proportional to channel width 22. Therefore, flow rates that are not constant because of the variations in channel width 22, contribute to band spreading beyond that of a normal parabolic flow profile of a liquid passing over a surface.

[0018] Collocated monolith structures 14 defining nearly vertical interconnected channels 12 are created by a variety of techniques. Suitable etching techniques, for example, include anisotropic etching techniques such as reactive ion etching, electron beam etching and LIGA (Lithographie Galvanoformung Abformung). These etching techniques are well known in the art. LIGA is a process that allows fabrication of three dimensional structures having high aspect ratios. The process involves four steps: irradiation, development, electroforming and resist stripping. Irradiation step involves irradiating a resist using laser, electron-beam or X-ray from a synchrotron radiation source. In the development step, a pattern is transferred into the resist and the resist is etched to reveal three dimensional structures comprising the resist material. In the electroforming step, a metallic mold is produced around the resist structures by electroplating. In the final step, the resist is stripped to reveal channels. Anisotropic wet etching may also be used to create the channels 12. Anisotropic wet etching, however, requires specific types of substrates. For example, the substrate must be crystalline and etching occurs along a specific axis.

[0019] In fabricating the separation column 10, first, a substrate 11 is provided to create microfabricated collocated monolith structures 14. Examples of materials suitable for substrates 11 include, but are not limited to, silicon, quartz, glass, and plastic. The substrate 11 is patterned to designate areas to be etched. The patterned substrate is etched to create collocated monolith support structures 14 and interconnected channels 12 defined by the support structures 14. In a preferred embodiment, the substrate 11 is etched by a process that provides channels 12 with uniform width.

[0020] Subsequent to etching the substrate 11, surfaces of the monolith structures 15 may be treated to provide interactions between the surfaces 15 and a sample passing through the separation column 11, thereby inducing separation of components of the sample. For example, surfaces of the monolith structures 15 may be coated with specific binding analytes by coating technologies known to or to be discovered by those skilled in the art. U.S. Patent No. 5,030,352, which describes a method of coating a surface of a separation column, is incorporated herein by reference. A coating technology for coating surfaces of the monolith structures 15 is not an aspect of the present invention. The coating may be thin or thick. Materials placed on the surfaces of the monolith structures 15, for example, include antibodies, cationic or anionic coatings, chelators, organic coatings including complex sugars and heparin, gels, fimbriae, and reverse phase coating such as C18. The specific binding analyte may be immobilized or entrapped in the channels 12.

[0021] In one embodiment, a cover plate 13 is added to create an enclosed separation column 10. The cover plate 13 may be attached by placing the cover plate 13 in contact with the etched surface of the substrate 11 and causing the cover plate 13 to bond to the etched

substrate 11. In the cases of a silica, glass or quartz cover plate 13, fusing creates cohesive bonding of very smooth surfaces. A cover plate 13 can be fused to the etched substrate 11 by allowing the two pieces to come in contact, placing them in an oven, and gradually raising the oven temperature. In some cases, bonding may take place at around 90°C. In other cases, the oven temperature may have to be raised up to 1000°C. Alternatively, for a silica or glass substrate, bonding may take place at room temperature by spinning on a layer of sodium silicate solution containing 5-7% solids and placing the substrate 11 and the cover plate 13 in contact. In another embodiment, a second etched substrate 11 having a mirror image of the first etched substrate 11 is disposed over and bonded to the first etched substrate forming a separation column with channel depth that is twice as long. In either case, a bonding process need not produce a continuous bond between the support structures 14 or the support structures 14 and the cover plate 13: However, the resulting bond must seal the channels 12 such that a solution inside the channels cannot communicate with the outside world along the interface of the two plates. Any other suitable bonding technique may be used without departing from the spirit of the present invention.

[0022] In application, a separation apparatus includes a plurality of reservoirs and at least one sample reservoir in communication with the separation column 11. In one embodiment, the separation apparatus includes a pump for pumping a mobile phase from a reservoir through the separation column 11. In another embodiment, the separation apparatus includes an electrophoresis apparatus in electrical communication with the separation column 11. The electrophoresis apparatus applies a potential across the separation column 11 for separating components of a sample passing through the separation column 11. In still another embodiment, the separation apparatus includes a detector in communication with the separation column 11 for detecting components separated by the separation column 11. The detector, for example, may be a mass spectrometer or an infrared detector. The operations of a mass spectrometer and an infrared detector are well known in the art. U.S. Patents 5,498,545 and 5,045,694, which describe mass spectrometers are incorporated herein by reference.

[0023] In the embodiment of Figures 1A and 1B, the collocated monolith support structures 14 have tetragonal cross-sectional areas 18. Tetragonal or hexagonal cross-sectional geometries are preferred over other geometries (e.g., triangular), because tetragonal or hexagonal geometries can create substantially rectangular interconnected channels 12 having high aspect ratios, as well as providing channels that are substantially parallel to the longitudinal axis of the separation column 16 when properly oriented. An aspect ratio is the ratio of the lengths of the depth to the width of a channel 12 between adjacent support structures 14, where the depth is the dimension perpendicular to the surface of

the substrate 11 and the width is the dimension perpendicular to the flow direction in the channel 12. Rectangular channels, as defined by a plane perpendicular to the substrate, having high aspect ratios, are preferred over traditional cylindrical channels for the following reasons. If a rectangular channel having a high aspect ratio (i.e., >> 5) and a traditional cylindrical channel with the same cross-sectional areas are used for liquid chromatography, the distance that a sample must travel to contact the maximum surface area of a stationary phase is shorter for the rectangular channel than it is for the traditional cylindrical channel. Likewise, if a rectangular channel and a cylindrical channel with same cross-sectional areas are used in electrokinetically driven separation systems, the distance that a heated solvent must travel to reach the maximum area of heat dispersing surface is shorter with a rectangular channel having a high aspect ratio than it is with a cylindrical channel. Channels that are substantially perpendicular to the longitudinal axis 16 of the separation column are not preferred, since they will be filled with stagnant pools of mobile phase and cause peak dispersion by the limitations of stagnant mobile phase mass transfer. This phenomenon is widely described in chromatographic systems packed with porous particles, which are filled with stagnant mobile phases. Tetragonal and hexagonal cross-sectional geometries are preferred, since they can provide channels that are substantially parallel, or at least not substantially perpendicular, to the longitudinal axis of the separation column 16, when properly oriented.

[0024] Other non-limiting, cross-sectional geometries for collocated monolith support structures 14 that create rectangular channels are shown in Figures 2A to 2F. Although columnar monolith structures having circular cross-sections may be created, they are less desirable than tetragonal or hexagonal geometries, because the intercolumnar channels created by the columnar structures will not be as uniform as those created by tetragonal or hexagonal geometries. The structures shown in the Figures 2A to 2F have the advantage in that they may be closely packed and still have uniform and controllable channel dimensions between monolith structures.

[0025] According to the invention, the interconnected channels 12 have an aspect ratio of greater than 5 and more preferably greater than 10. Greater aspect ratios are possible by etching the substrate 11 deeper. The channel width is generally in the range of 1-10 μm for chromatography applications. Although a separation column 10 having channel widths of less than 1 μm may be desirable to reduce band broadening in chromatography, other operational problems such as plugging and high pressure requirements exist with such narrow columns.

[0026] In a preferred embodiment, the separation column 10 has a first group and a second group of channels 12, where the channels in each group are parallel to each other and the channels in the first group intersect

with the channels in the second group. Where the channels 12 intersect, the point of intersection preferably is deeper than it is wide.

[0027] Determination of the depth and the aspect ratio of a channel 12 involves a compromise. A longer channel depth is useful in pressure driven separation systems, because mobile phase volume is increased allowing more sample to be carried in a channel. However, in electrically driven separation systems, heat transfer becomes limited with a longer channel depth. When operating at high voltage, joule heating causes transaxial thermal gradients to develop along the depth of channels having high aspect ratios. In a dense channel system where the aspect ratio of each channel goes beyond 10-20 and the channel depth is greater than 20 μm, heat transfer to the surface of the chip can become limiting, unless channel density is decreased.

[0028] Determination of the channel width also involves a compromise. Channels 12 having widths smaller than 1-2 μm increase transfer rate of sample components to the channel surfaces where the components can interact with the surface. However, in a pressure driven system, the operating pressure for a separation column 10 with such narrow channels 12 is large, making it difficult to get liquid into the channel network, and more susceptible to plugging.

[0029] In one embodiment, the substrate 11 is etched to create interconnected channels having an aspect ratio of from about 5 to about 100. Even higher aspect ratios may be desirable, but is beyond the limits of current microfabrication technology. In a preferred embodiment, the aspect ratio of a channel 12 in a voltage driven separation system is from about 10 to about 20, whereas the aspect ratio of a channel 12 in a pressure driven separation system is greater than 20. Current typical microfabrication technology allows resolution in the production of masks and etching to about 0.1 μm. Therefore, the lower limitation on a channel width is approximately 0.5 ± 0.1 μm and the upper limitation on the depth of such channel is approximately 10 μm in chromatographic systems. Separation columns having channels of such dimensions, fabricated with existing technologies, however, can exhibit channel heterogeneity, which leads to peak dispersion. Channel heterogeneity, however, is caused by fabrication limitations and not design, and therefore is expected to improve as fabrication technologies advance.

[0030] According to the present invention, geometry and size of the collocated monolith structures 14 and the interconnected channels 12 may be selected to optimize specific functions. For example, in one embodiment, separation columns are designed to optimize interchannel coupling. Interchannel coupling refers to mixing of streams from multiple channels to average heterogeneity in flow and peak dispersion between individual channels across many channels. The dominant concern with multi-channel systems is that the channels may not be identical in terms of migration velocity and

fluid dynamics. The separation columns of the present invention overcome this concern by mixing fluid from adjacent channels at periodic intervals along the length of the separation system.

[0031] Figures 2A to 2F show examples of collocated monolith support structures 30, 34, 38, 42, 46, 50 for achieving interchannel coupling. The illustrations in Figures 2A to 2F suggest that streams from adjacent channels will completely merge and mix, then spread laterally at the channel junctions 31, 35, 39, 43, 47, 51 into downstream channels. However, in reality, incomplete mixing is likely at high mobile phase velocity. Three types of interchannel geometry to achieve intercoupling are revealed in Figures 2A to 2F. Figures 2A, 2B and 2C show a (Y) shape configuration for interchannel coupling, Figures 2D and 2E show an (X) shape configuration for interchannel coupling, and Figure 2F shows a (T) shape configuration for interchannel coupling. The T shape configuration in Figure 2F may be used to achieve interchannel coupling, but is not a preferred geometry. It has been observed that since adjacent channels in the T shape configuration intersection in a horizontal line and not a point, a non-streamline flow results Stagnant pools of liquid 51, 52 in the channel adjacent a bottom surface 53 of a monolith structure 50 and a top surface 53' of a monolith structure 50' accumulate. The bottom surface 53 is the surface of a monolith structure 50 perpendicular to the flow direction 55 and the last surface of the monolith 50 to come in contact with a component passing through the column. The top surface 53' is the surface of a monolith structure 50' also perpendicular to the flow direction 55 and the first surface of the monolith 50' to come in contact with the component passing through the column. Samples will diffuse into and out of these stagnant pools, and in the course of doing so, band spreading will result. In a preferred embodiment, monolith support structures 30, 34, 38, 42, 46 define channels 31, 35, 39, 43, 47 that intersect in X or Y shape configurations.

[0032] The geometry of monolith support structures affects interchannel coupling in another manner. Monolith support structures 30, 34 shown in Figures 2A and 2B result in less interchannel coupling than monolith support structures 38, 42 shown in Figures 2C and 2D. Assuming that two separation columns have equal length with one column having support structures 30 shown in Figure 2A and the other having support structures 38 shown in Figure 2C, a sample in the first column 32 must travel further before intercoupling and encounters slightly fewer opportunities for intercoupling. This is because the support structures 30 in Figures 2A are elongated as compared to the support structures 38 in Figure 2C. The monolith geometries 30, 34 represented in Figures 2A and 2B are preferred when the degree of channel homogeneity is high, such that not much interchannel coupling is required. On the other hand, when there are interchannel differences in either the rate of flow or peak dispersion caused by faulty fabrication,

fouling during operation, leaching of organic surface coatings, or some other type of aging, structures similar to the ones shown in Figures 2C and 2D are preferred because they provide more interchannel coupling. The monolith structures 30, 34 in Figures 2A and 2B have a length that is substantially longer than the width. The monolith structures 38, 42 in Figures 2C and 2D, have a length that is substantially equal to the width. The length is the dimension parallel to the longitudinal axis 55 of the separation column and the width is the dimension perpendicular to the longitudinal axis 55 of the separation column, where both dimensions are parallel to the surface of the substrate. The net effect of interchannel coupling is that heterogeneity between channels can be "averaged" or distributed across many channels as a sample migrates through the system.

[0033] In another embodiment, referring to Figure 2E, a separation column 48 is designed to increase separation efficiency per unit length of the column 48. Separation efficiency is increased by creating monolith structures 46 and channels 49 that provide greater lateral migration relative to longitudinal migration between inter-coupling. The monolith structures 46 have a length that is substantially shorter than the width. The net effect is that the migration distance of a sample through a longitudinal unit length of this column 48 is increased. This embodiment has properties similar to the serpentine channel columns existing in the prior art. Serpentine channel columns are used to increase the migration distance of a sample within the limited space available on a chip. The problem with the serpentine channel approach is the "race track" effect caused by the corners. The "race track" effect refers to the effect of components of a sample traveling near the inner surface of the corner covering a shorter distance than components traveling near the outer surface of the corner. The difference in distance covered can add to zone broadening. The great advantage of the embodiment shown in Figure 2E is that it accomplishes efficiency per unit length but by using multiple channels and interchannel coupling, overcomes the "race track" effect. The embodiment of Figure 2E also provides larger capacity with channels of the same width.

[0034] In yet another embodiment, the separation column of the invention is designed to maximize heat dissipation. An electrophoretic current applied to a separation column causes joule heating. Joule heating contributes to band spreading by creating thermal gradients, which produce transchannel convection. According to the present invention, heat is dissipated through the monolith support structures, which are in communication with a substrate or a cover at both ends. The separation columns of the present invention maximizes heat dissipation in the following ways. First, heat dissipation is maximized by creating collocated monolith support structures and interchannels with high surface to volume ratio. Surface area refers to total wall space adjacent a single channel. Volume refers to volume of

a single channel. Second, monolith structure mass to channel volume ratio is increased. Third, channel density in a separation column is minimized. Channel density can be minimized through monolith geometry. In a preferred embodiment, monolith structures with square cross-sectional areas are used to minimize channel density. Finally, channel height is minimized.

[0035] Narrow channels between monoliths having tetragonal and hexagonal cross-sections are generally suitable for heat dissipation, as they provide both a large surface area to liquid volume ratio per channel and a low density of channels distributed throughout a column. Referring to Figures 3A and 3C, narrow channels 60, 62 between tetragonal monoliths 64 and hexagonal monoliths 66 meet these criterion. However, the monolith geometry 66 shown in Figure 3A is preferred over the monolith geometry 68 shown in Figure 3B, because the monolith geometry 66 in Figure 3A provides lower channel density and higher monolith mass to channel surface area than the monolith geometry 68 shown in Figure 3D. The monolith 70 shown in Figure 3D is preferred over the monolith 64 shown in Figure 3C for the same reasons.

[0036] In yet another embodiment, separation columns are designed to minimize band spreading caused by a parabolic velocity distribution of a solution passing through the column. Parabolic velocity distribution in liquid chromatography becomes worse as the width of the separation column increases. The distance between adjacent support structures is minimized to reduce band spreading without encountering operational problems. In a preferred embodiment, the minimum channel width is about 1 μm.

[0037] In yet another embodiment, separation columns in which the flow is electroosmotically driven are designed to minimize flow heterogeneity. Electroosmotic flow (EOF) refers to movement of liquid inside a separation column due to application of an electric field. The velocity of electroosmotic flow is related to a zeta potential generated at the surface of the column, the dielectric constant of the solution and the viscosity of the double layer formed at the surface of the column. Although there are localized regions of inhomogeneity in zeta potential, EOF in a 10-100 cm open tubular capillary is relatively uniform. Referring to Figures 4A and 4B, EOF in a collocated monolith support structure system 72 differs from that in a single open tubular capillary. For example, there is the difference in lateral (or radial) electrical potential. Maximum electrical potential will be found where the field lines take the shortest route between the system electrodes. The electric potential is thought to be uniform across the separation channel in a single, long, open tubular capillary for this reason. In contrast, the shortest route between the electrodes in a collocated monolith support structure system 72 shown in Figures 4A and 4B is to cut diagonally across channels 75 that are not parallel with the electric field 76 in the system. Because there is a slightly higher potential

on one side of the channel 75, it is expected that EOF on that face of the channel will be higher. In a system operating at 1000 V/cm (0.1 V/μm), there is a potential drop of approximately one volt along the length of a channel that is 10 μm long. A channel length is a dimension parallel to the surface of the substrate and parallel to the direction of flow inside the channel. It is seen in Figures 4A and 4B that the diagonal nature of the channels can cause a vertical voltage differential of 88-350 mV at the positions 73 and 74 of individual channels 75. It is probable that this diagonal field effect will induce flow heterogeneity within channels 75, which could impact interchannel coupling at the channel junctions. The diagonal field line effect is greater in the separation column having wider channels 75 shown in Figure 4A and less in the separation column having narrower channels 77 shown in Figure 4B. In a preferred embodiment, the separation column driven by electroosmotic flow has collocated monolith support structures defining long, narrow channels 77 as shown in Figure 4B to minimize the diagonal field line effect.

[0038] In still another embodiment, separation columns are designed to maximize the ratio of the overall surface areas of the support structures to the overall volume of the channels, defined as the A/V ratio. In chromatography, increasing the A/V ratio is advantageous as it increases the phase ratio and loading capacity. Phase ratio is the ratio of the area of the surface on which the stationary phase is supported to the volume of the mobile phase. When the phase ratio is very small, components of a sample are not adequately retained to achieve separation and resolution. In electrophoresis, separation columns with an high A/V ratio dissipates heat caused by joule heating with greater efficiency.

[0039] According to the invention, the A/V ratio is maximized by making the channels as long as possible and the channel width as narrow as possible, and minimizing the number of channel junctions. A single, long capillary would be ideal to maximize the A/V ratio, but there are other overriding advantages to multi-channel systems. Acceptable limits on the A/V ratio should not compromise other variables in the system. According to the invention, the channel length (1) to width (w) ratio exceeds 3 and preferably exceeds 5.

[0040] In still another embodiment, separation columns are designed to eliminate "wall effects". "Wall effects" refer to the potential for stagnant pools of liquid to form at the walls of separation columns comprising collocated monolith support structures. Referring to Figure 5A, stagnant pools 80 of liquid can form between a wall 82 and a corner of a tetragonal monolith 84. In some respect, this is similar to the "race track" effect noted above and may contribute to peak dispersion. In the embodiment of Figure 5C, the monolith geometry 86 eliminates the potential for any dead spaces at the walls 88 such that the walls 88 are swept by the liquid flow. A hexagonal monolith 86 geometry allows a flat side of a monolith 87 to be parallel to the wall 88 such that there

is no dead space between the monolith 86 and the wall 88. At the same time, the hexagonal monolith 86 geometry provides interchannel coupling by having a Y-shaped channel configuration 90. In the embodiment of Figure 5B, rounding the corners during etching process eliminates dead spaces 80 (shown in Figure 5A) between the walls 92 and the corners of the tetragonal monoliths 94 and thereby also eliminating the "wall effects".

[0041] In prior art chromatography columns, the diameter of a column is many times larger (frequently >10X) than the diameter of the inlet or the outlet channel of the column. This presents several challenges. One challenge is to homogeneously distribute mobile phase and analyte laterally across the head of the column at the inlet without creating band spreading. Another challenge is to homogeneously collect the mobile phase and the analyte after they have traversed the length of the column without causing zonal dispersion. In packed microcolumns, this is frequently achieved by fusing microparticle silica particles at the column outlet. This process is very similar to the fusion process used to produce the "frit" in a fritted glass filter funnel. The problem with this approach is that it is very difficult to pack these particles uniformly and then fuse them inside the capillary. The "fused frit" approach has been reported to cause serious zonal dispersion because they are not uniform causing flow inhomogeneity.

[0042] Referring to Figure 6, the present invention eliminates the need for column terminating frits because the monolith supports, which take the place of particles, are all fabricated on a single wafer and therefore are immobilized. However, there is the issue of distributing and collecting the mobile phase at the column ends 114. The invention addresses the fluid mechanics of homogeneously splitting and combining streams at the ends of separation columns 100, 102 by creating a collocated monolith distributor at an entry end of a separation column and a collocated monolith collector at an exit end of the separation column. In a multi-dimensional system, each separation column may comprise a collocated monolith distributor and a collocated monolith collector.

[0043] The concept behind the collocated monolith distributor 96 is to use monolith structures 97 to create a channel network 99, which sequentially splits a single channel into multiple channels by X^n factor, where X is the number of channels that a single channel splits into and n is the number of times splitting takes place to provide communication between the channels 101 in the channel network 99 and the channels 104 in the separation column 100. In the inlet 96 disclosed in Figure 6, a single stream 106 is first homogeneously split into two streams 107, the two streams 107 are split into four streams 108, the four streams 108 are split into eight streams 109, etc. The total number of channels (C) laterally across the inlet channel network 99 of the distributor 96 shown in the figure can be expressed by the

equation

$$C=2^n$$

where n is the number of times the liquid stream splits. In a preferred embodiment, the channels 101 in the inlet channel network 99 splits by 2^n factor. Although it is possible to use splitting systems that follow 3^n, 4^n, or X^n rule, it is more difficult to keep the path length of all channels equal without increasing tortuosity in some channels. However, these structures may be preferred in cases where a wider column layout is needed for higher sample capacity. With these embodiments, constant cross-sectional areas of channels are maintained by the addition of two monolith structures in between the channels.

[0044] Interchannel splitting provided by the inlet channel network 99 causes the same volume of liquid to reach all points in a lateral cross-section of the separation column 100 at the same time. Any system which causes this to happen will give homogeneous interchannel splitting in the delivery of the mobile phase and sample separation column. In a preferred embodiment, the inlet channel network 101 has channels of equal width, height, and length to achieve homogeneous interchannel splitting. In another embodiment, where the inlet channel network 99 has channels 101 with differing length and width, the length and width of each channel is adjusted such that equal volumes of liquid reach all points at the column inlet to maintain homogeneous interchannel splitting.

[0045] In one embodiment, cross-sectional areas of all channels 101 in the inlet channel network 99 are substantially equal. The cross-section area of a channel is perpendicular to the longitudinal axis 110 of the separation column An advantage of this embodiment is that narrow channels 101 used throughout the network 99 minimizes "race-track" effects in channels that provide corners. Disadvantages of this embodiment are that liquid flowing into the separation column 100 have non-uniform velocities, which can cause zone broadening and increase degassing (bubble formation) from mobile phases in EOF pumped columns. Since all channels 101 are the same width, the total cross-sectional area of the channels double at each level of splitting in the 2^n system. The linear velocity of the mobile phase slows down as the mobile phase passes through subsequently split channels, since velocity is inversely proportion to cross-sectional area. Further more, the pressure will vary inversely with cross-sectional area.

[0046] In the embodiment shown in Figure 6, the cross-sectional areas of the channels 101 in the inlet channel network 99 are sequentially halved as the number of channels 101 in the network 99 double at each level of splitting. This embodiment maintains the total cross-sectional area of the network across all planes, measured orthogonal to the longitudinal axis

110 of the separation column 100 to be substantially constant. Furthermore, channels at each split level have the same cross-sectional area. Advantages of this embodiment are that linear velocity of mobile phase and pressure drop are constant at all points in the system.

[0047] The monolith collector 98 is created in a manner similar to the monolith distributor 96. Adjacent channels 116 in the network 118 are sequentially combined by X^n factor, where X is the number of adjacent channels 116 that combine into a single channel and n is the number of times combinations take place. Combinations take place until all channels are combined into a single column 112.

[0048] Therefore, a monolith distributor 96 or a monolith collector 98 having all channels with equal cross-sectional areas is preferred when the objective is to minimize intracolumn zonal dispersion, i.e., no "race-track" effect, whereas a monolith distributor 96 or a monolith collector 98 with constant total cross-sectional area for channels in the same split level is preferred when the objective is to minimize extra column zonal dispersion, i.e., constant velocity and pressure.

[0049] Figure 7 shows an electropherogram of electrophoretic separation of Rhodamine and Fluorescein using a separation apparatus of the present invention. Figure 8 shows an electropherogram of electrophoretic separation of peptides from human growth hormone (HGH) using a separation apparatus of the present invention. The separation column of the separation apparatus used to perform the separations has a plate height of approximately one micron.

Claims

1.   A separation column (10;100,102) comprising:

a substrate (11);
a longitudinal axis (16;110);
a plurality of collocated monolith support structures (2;14;30;34;38;42;46;50,50';64;66;68;70;84;86;94;97) formed on said substrate (11) and arrayed in two dimensions to define a plurality of interconnected channels (6;12;31;32;35;39;43;47;49;60;62;75;77;90;104,108,109); and
an inlet (96) and/or outlet (98) comprising a channel network (99;118) that sequentially splits a single channel (112;114) into multiple channels (101;116) by X^n where "X" is the number of channels into which a single channel splits and "n" is the number of times the channels split along the longitudinal axis (16;110), wherein the channel network (99;118) of the inlet/outlet (96;98) is defined by monolith structures formed on said substrate (11) and is in fluid communication with said plurality of interconnected channels (6;12;31;32;35;39;43;47;49;

60;62;75;77;90;104,108,109).

2. The separation column of claim 1 wherein each support structure (14) of the plurality of collocated monolith support structures (14) has a first end (7) and a second end (9), and the plurality of collocated monolith support structures (14) are connected with each other at the first end (7) of each collocated monolith support structure (14).

3. The separation column of claim 2 further comprising a cover plate (13) connected with each of the plurality of collocated monolith support structures (14) at the second end (9) of each monolith support structure (14).

4. The separation column of claim 2 wherein the plurality of collocated monolith support structures (2,14) comprises a first and a second group of collocated monolith support structures, the second group being a mirror image of the first group and disposed over the first group, such that the second end (9) of each monolith support structure (14) of the first group is in communication with the second end (4) of a corresponding monolith support structure (2) of the second group, thereby closing the separation column.

5. The separation column of any one of claims 1 to 4 wherein the plurality of collocated monolith support structures (2;14) have surfaces (1;15) capable of interacting with analytes passing through the plurality of interconnected channels (6;12) to induce separation of the analytes.

6. The separation column of claim 5 wherein the surfaces (1;15) of the monolith support structures (2; 14) are coated with a specific binding analyte.

7. The separation column of any one of claims 1 to 6 wherein the plurality of interconnected channels (6; 12;31;32;35;39;43;47;49;60;62;75;77;90; 104,108,109) are substantially uniform in shape and size.

8. The separation column of any one of claims 1 to 7 wherein the plurality of interconnected channels are substantially rectangular channels.

9. The separation column of any one of claims 1 to 8 wherein each of the plurality of collocated monolith support structures (2;14;38;42;50,50';64;70;84;94; 97) has a tetragonal cross-section, wherein the cross-section is a plane parallel to a plane of a substrate (11) supporting the support structures.

10. The separation column of any one of claims 1 to 8 wherein each of the plurality of collocated monolith

support structures (30;34;66;68;86) has a hexagonal cross-section, wherein the cross-section is a plane parallel to a plane of a substrate (11) supporting the support structures.

11. The separation column of claim 8 or 9 wherein an aspect ratio of a channel between two adjacent support structures is in the range of from about 5 to about 100, wherein the aspect ratio is the ratio of a depth to a width of a channel, where the depth is a dimension perpendicular to a plane of the substrate (11) supporting the support structures and the width is a dimension perpendicular to a flow direction in the channel and parallel to the plane of the substrate (11).

12. The separation column of any one of claims 1 to 11 wherein interchannel coupling is provided by the plurality of interconnected channels defined by the plurality of collocated monolith support structures, each collocated monolith structure having a length that is substantially equal to a width where the length is a dimension parallel to and the width is a dimension perpendicular to a longitudinal axis of the separation column and parallel to a plane of the substrate.

13. The separation column of any one of claims 1 to 12 wherein interchannel coupling is provided by X or Y shape configuration of the intersecting channels.

14. The separation column of any one of claims 1 to 13 wherein an electrophoretic current is adapted to be applied to the column and heat created by the current is dissipated through the plurality of monolith support structures.

15. The separation column of any one of claims 1 to 14 wherein each of the monolith support structures is sized to maximize heat dissipation.

16. The separation column of any one of claims 1 to 14 wherein separation efficiency per unit length of the separation column is provided by the plurality of collocated monolith support structures, a cross sectional area of each monolith support structure of the plurality of collocated support structures measured parallel to a substrate supporting the support structures having a length that is substantially shorter than a width, wherein the length is parallel to a longitudinal axis of the separation column and the width is substantially perpendicular to the longitudinal axis of the separation column and parallel to a plane of the substrate.

17. The separation column of any one of claims 1 to 16 wherein the channels adjacent walls (88) of the separation column are substantially parallel to the walls

thereby to substantially eliminate a wall-effect.

18. The separation column of any one of claims 1 to 16 wherein corners of the channels adjacent walls (92) of the separation columns (94) are rounded to eliminate any dead space (80).

19. The separation column of any one of claims 1 to 18 wherein cross-sectional areas of all of the channels (101;116) in the channel network (99;118) are substantially equal.

20. The separation column of any one of claims 1 to 18 wherein cross-sectional areas of the channels (101; 116) in the channel network (99;118) are sequentially reduced as the number of channels (101;116) in the channel network (99;118) increases, thereby maintaining the total cross sectional area of the channel network across all planes orthogonal to the longitudinal axis (16;110) of the separation column to be substantially constant.

21. The separation column of any one of claims 1 to 20, wherein the plurality of interconnected channels (6; 12;31;32;35;39;43;47;49;60;62;75;77;90; 104,108,109) are defined by the plurality of collocated monolith support structures (2;14;30;34;38; 42;46;50,50';64;66;68;70;84;86;94;97) such that they sequentially merge and split.

22. A method for manufacturing the separation column of any one of claims 1 to 21, the method comprising:

   providing a substrate (11);
   patterning the substrate (11) to designate areas of the substrate (11) to be etched; -
   etching the substrate (11) to create the plurality of collocated monolith support structures (2;14) defining the plurality of interconnected channels and the monolith structures creating the channel network of the inlet (96) and/or outlet (98); and
   closing the separation column.

23. The method of claim 22 wherein

   closing the separation column comprises attaching a cover plate (13) on a surface of the plurality of collocated monolith support structures (14).

24. The method of claim 22 wherein

   providing the substrate comprises providing a first and a second substrate;
   etching the substrate comprises etching the first and the second substrate to create the collocated monolith support structures (2,14) and

the inlet (96) and/or outlet (98) on the first and the second substrate that are mirror images; and
closing the separation column comprises disposing the second substrate over the first substrate such that each monolith support structure (14) of the first substrate is in communication with a corresponding monolith support structure (2) of the second substrate, thereby closing the separation column.

25. The method of claim 22, 23 or 24 wherein etching the substrate comprises anisotropic etching, LIGA etching, reactive ion etching, or electron beam etching.

26. A separation apparatus comprising:

   a separation column of any one of claims 1 to 21;
   a plurality of reservoirs in communication with the separation column; and
   a sample reservoir in communication with the separation column.

27. The separation apparatus of claim 26 further comprising a pump located to pump a mobile phase from a reservoir through the separation column.

28. The separation apparatus of claim 26 further comprising an electrophoresis apparatus in electrical communication with the separation column, the electrophoresis apparatus applying a potential across the separation column for separating components of a sample passing through the separation column.

29. The separation apparatus of claim 26 further comprising a detector in communication with the separation column.

30. The separation apparatus of claim 29 wherein the detector is a mass spectrometer.

31. A method for separating components of a sample, the method comprising:

   introducing a medium solution into the inlet of the separation column of any one of claims 1 to 21;
   introducing a sample into the inlet of the separation column; and
   separating components of the sample as the sample passes through the separation column.

32. The method of claim 31 wherein separating the components of the sample comprises applying a potential to the column such that components of the

sample are separated by electrophoretic mobility.

Patentansprüche

1. Trennsäule (10;100,102) mit:

   einem Substrat (11),
   einer Longitudinalachse (16;110),
   mehreren nebeneinander angeordneten Monolith-Halterungsstrukturen (2;14;30;34;38;42; 46;50,50';64;66;68; 70;84;86;94;97), die auf dem Substrat (11) ausgebildet sind und in zwei Dimensionen nebeneinander angeordnet sind, um mehrere miteinander verbundene Kanäle (6;12;31;32;35;39;43; 47;49;60;62;75;77;90; 104,108,109) festzulegen, und
   einem Einlaß (96) und/oder Auslaß (98) mit einem Kanalnetz (99;118), welches sequentiell einen einzelnen Kanal (112;114) in mehrere Kanäle (101;116) durch X^n unterteilt, wobei "X" die Anzahl von Kanälen ist, in die ein einzelner Kanal unterteilt ist, und "n" die Anzahl von Malen ist, die die Kanäle entlang der Longitudinalachse (16;110) unterteilt sind, wobei das Kanalnetz (99;118) des Einlasses/Auslasses (96; 98) durch auf dem Substrat (11) ausgebildete Monolithstrukturen festgelegt ist und in Fluidverbindung mit den mehreren miteinander verbundenen Kanälen (6;12;31;32;35; 39;43;47; 49;60;62;75;77;90;104,108,109) steht.

2. Trennsäule nach Anspruch 1, wobei jede Halterungsstruktur (14) der mehreren nebeneinander angeordneten Monolith-Halterungsstrukturen (14) ein erstes Ende (7) und ein zweites Ende (9) aufweist und die mehreren nebeneinander angeordneten Monolith-Halterungsstrukturen (14) miteinander am ersten Ende (7) jeder nebeneinander angeordneten Monolith-Halterungsstruktur (14) verbunden sind.

3. Trennsäule nach Anspruch 2, ferner mit einer Abdeckplatte (13), die mit jeder der mehreren nebeneinander angeordneten Monolith-Halterungsstrukturen (14) am zweiten Ende (9) jeder Monolith-Halterungsstruktur (14) verbunden ist.

4. Trennsäule nach Anspruch 2, wobei die mehreren nebeneinander angeordneten Monolith-Halterungsstrukturen (2,14) eine erste und eine zweite Gruppe nebeneinander angeordneter Monolith-Halterungsstrukturen umfaßt, wobei die zweite Gruppe ein Spiegelbild der ersten Gruppe ist und über der ersten Gruppe so nebeneinander angeordnet ist, dass das zweite Ende (9) jeder Monolith-Halterungsstruktur (14) der ersten Gruppe in Verbindung mit dem zweiten Ende (4) einer entsprechenden Monolith-Halterungsstruktur (2) der zweiten Gruppe steht, wodurch die Trennsäule geschlossen ist.

5. Trennsäule nach einem der Ansprüche 1 bis 4, wobei die mehreren nebeneinander angeordneten Monolith-Halterungsstrukturen (2;14) Oberflächen (1;15) aufweisen, die mit durch die mehreren miteinander verbundenen Kanäle (6;12) durchlaufenden Analyten in Interaktion treten können, um eine Trennung der Analyten zu induzieren.

6. Trennsäule nach Anspruch 5, wobei die Oberflächen (1;15) der Monolith-Halterungsstrukturen (2; 14) mit einem spezifischen Bindungsanalyten beschichtet sind.

7. Trennsäule nach einem der Ansprüche 1 bis 6, wobei die mehreren untereinander verbundenen Kanäle (6;12;31;32;35;39; 43;47;49;60;62;75;77;90; 104,108,109) in der Form und der Größe im wesentlichen gleichmäßig sind.

8. Trennsäule nach einem der Ansprüche 1 bis 7, wobei die mehreren untereinander verbundenen Kanäle im wesentlichen rechteckige Kanäle sind.

9. Trennsäule nach einem der Ansprüche 1 bis 8, wobei jede der mehreren nebeneinander angeordneten Monolith-Halterungsstrukturen (2;14;38;42; 50,50';64;70;84;94;97) einen tetragonalen Querschnitt aufweist, wobei der Querschnitt eine zu einer Ebene eines die Halterungsstrukturen tragenden Substrats (11) parallele Ebene ist.

10. Trennsäule nach einem der Ansprüche 1 bis 8, wobei jede der mehreren nebeneinander angeordneten Monolith-Halterungsstrukturen (30;34;66;68; 86) einen hexagonalen Querschnitt aufweist, wobei der Querschnitt eine zu einer Ebene eines die Halterungsstrukturen tragenden Substrats (11) parallele Ebene ist.

11. Trennsäule nach Anspruch 8 oder 9, wobei ein Seitenverhältnis eines Kanals zwischen zwei aneinandergrenzenden Halterungsstrukturen im Bereich von etwa 5 bis etwa 100 liegt, wobei das Seitenverhältnis das Verhältnis einer Tiefe zu einer Breite eines Kanals ist, bei dem die Tiefe eine Dimension senkrecht zu einer Ebene des die Halterungsstrukturen tragenden Substrats (11) ist und die Breite eine Dimension senkrecht zu einer Strömungsrichtung in dem Kanal und parallel zu der Ebene des Substrats (11) ist.

12. Trennsäule nach einem der Ansprüche 1 bis 11, wobei eine Zwischenkanalkoppelung durch die mehreren, mittels der mehreren nebeneinander ange-

ordneten Monolith-Halterungsstrukturen festgeleg-
ten, miteinander verbundenen Kanäle vorgesehen
ist, wobei jede nebeneinander angeordnete Mono-
lithstruktur eine Länge aufweist, die im wesentli-
chen gleich einer Breite ist, bei der die Länge eine
Dimension parallel zu, und die Breite eine Dimen-
sion senkrecht zu einer Longitudinalachse der
Trennsäule und parallel zu einer Ebene des Sub-
strats ist.

13. Trennsäule nach einem der Ansprüche 1 bis 12, wo-
bei eine Zwischenkanalkoppelung durch eine X-
oder Y-förmige Konfiguration der sich kreuzenden
Kanäle gebildet ist.

14. Trennsäule nach einem der Ansprüche 1 bis 13, wo-
bei ein elektrophoretischer Strom an die Säule an-
gelegt werden kann und durch den Strom erzeugte
Wärme über die mehreren Monolith-Halterungs-
strukturen zerstreut bzw. abgeleitet wird.

15. Trennsäule nach einem der Ansprüche 1 bis 14, wo-
bei jede der Monolith-Halterungsstrukturen so be-
messen ist, dass sie eine Wärmeableitung maxi-
miert.

16. Trennsäule nach einem der Ansprüche 1 bis 14, wo-
bei die Trenneffizienz pro Längeneinheit der Trenn-
säule durch die mehreren nebeneinander angeord-
neten Monolith-Halterungsstrukturen bereitgestellt
wird, wobei eine Querschnittsfläche jeder Monolith-
Halterungsstruktur der mehreren nebeneinander
angeordneten Halterungsstrukturen, parallel zu ei-
nem die Halterungsstrukturen tragenden Substrat
gemessen, eine Länge aufweist, die wesentlich kür-
zer als eine Breite ist, wobei die Länge parallel zu
einer Longitudinalachse der Trennsäule ist und die
Breite im wesentlichen senkrecht zu der Longitudi-
nalachse der Trennsäule und parallel zu einer Ebe-
ne des Substrats ist.

17. Trennsäule nach einem der Ansprüche 1 bis 16, wo-
bei die Kanäle angrenzend an Wände (88) der
Trennsäule im wesentlichen parallel zu den Wän-
den sind, wodurch ein Wandeffekt im wesentlichen
eliminiert wird.

18. Trennsäule nach einem der Ansprüche 1 bis 16, wo-
bei Ecken der Kanäle angrenzend an Wände (92)
der Trennsäulen (94) abgerundet sind, um jeglichen
Totraum (80) zu eliminieren.

19. Trennsäule nach einem der Ansprüche 1 bis 18, wo-
bei die Querschnittsflächen aller Kanäle (101;116)
in dem Kanalnetz (99;118) im wesentlichen gleich
sind.

20. Trennsäule nach einem der Ansprüche 1 bis 18, wo-

bei die Querschnittsflächen der Kanäle (101;116) in
dem Kanalnetz (99;118) sequentiell mit zunehmen-
der Anzahl von Kanälen (101;116) in dem Kanal-
netz (99;118) geringer sind, wodurch die Gesamt-
querschnittsfläche des Kanalnetzes über allen zu
der Longitudinalachse (16;110) der Trennsäule or-
thogonalen Ebenen beibehalten wird, so dass sie
im wesentlichen konstant ist.

21. Trennsäule nach einem der Ansprüche 1 bis 20, wo-
bei die mehreren miteinander verbundenen Kanäle
(6;12;31;32;35;39;43;   47;49;60;62;75;77;90;
104,108,109) durch die mehreren nebeneinander
angeordneten Monolith-Halterungsstrukturen (2;
14;30;34;38;42;46;50,50';64;66;68;70;84;86;94;
97) festgelegt sind, so dass sie sequentiell ineinan-
der übergehen und sich aufteilen.

22. Verfahren zur Herstellung der Trennsäule nach ei-
nem der Ansprüche 1 bis 21, wobei das Verfahren
umfasst:

      Bereitstellen eines Substrats (11),
      Strukturieren des Substrats (11), um Flächen
      des Substrats (11), die zu ätzen sind, zu be-
      stimmen,
      Ätzen des Substrats (11), um die mehreren ne-
      beneinander angeordneten Monolith-Halte-
      rungsstrukturen (2;14) zu erzeugen, welche die
      mehreren miteinander verbundenen Kanäle
      festlegen, wobei die Monolithstrukturen das
      Kanalnetz des Einlasses (96) und/oder des
      Auslasses (98) erzeugen; und
      Schließen der Trennsäule.

23. Verfahren nach Anspruch 22, wobei
      das Schließen der Trennsäule das Anbringen
      einer Abdeckplatte (13) auf einer Oberfläche der
      mehreren nebeneinander angeordneten Monolith-
      Halterungsstrukturen (14) umfasst.

24. Verfahren nach Anspruch 22, wobei
      das Bereitstellen des Substrats das Bereit-
      stellen eines ersten und zweiten Substrats umfasst,
      das Ätzen des Substrats das Ätzen des ersten
      und zweiten Substrats umfasst, um die nebenein-
      ander angeordneten Monolith-Halterungsstruktu-
      ren (2,14) und den Einlaß (96) und/oder Auslaß (98)
      auf dem ersten und dem zweiten Substrat, die Spie-
      gelbilder sind, zu erzeugen, und
      das Schließen der Trennsäule das Anordnen
      des zweiten Substrats über dem ersten Substrat
      umfasst, so dass jede Monolith-Halterungsstruktur
      (14) des ersten Substrats in Verbindung mit einer
      entsprechenden Monolith-Halterungsstruktur (2)
      des zweiten Substrats steht, wodurch die Trenn-
      säule geschlossen wird.

25. Verfahren nach Anspruch 22, 23 oder 24, wobei das Ätzen des Substrats ein anisotropes Ätzen, ein LI-GA-Ätzen, ein reaktives Ionenätzen oder ein Elektronenstrahlätzen umfasst.

26. Trennvorrichtung mit:

einer Trennsäule nach einem der Ansprüche 1 bis 21, mehreren Behältern in Verbindung mit der Trennsäule, und
einem Probenbehälter in Verbindung mit der Trennsäule.

27. Trennvorrichtung nach Anspruch 26, ferner mit einer Pumpe, die so angeordnet ist, dass sie eine mobile Phase aus einem Behälter durch die Trennsäule pumpt.

28. Trennvorrichtung nach Anspruch 26, ferner mit einer Elektrophorese-Vorrichtung in elektrischer Verbindung mit der Trennsäule, wobei die Elektrophorese-Vorrichtung ein Potential über der Trennsäule zum Trennen von Komponenten einer die Trennsäule durchlaufenden Probe anlegt.

29. Trennvorrichtung nach Anspruch 26, ferner mit einem Detektor in Verbindung mit der Trennsäule.

30. Trennvorrichtung nach Anspruch 29, wobei der Detektor ein Massenspektrometer ist.

31. Verfahren zum Trennen von Komponenten einer Probe, wobei das Verfahren umfasst:

Einbringen einer Mediumlösung in den Einlaß der Trennsäule nach einem der Ansprüche 1 bis 21,
Einbringen einer Probe in den Einlaß der Trennsäule, und
Trennen von Komponenten der Probe, wenn die Probe die Trennsäule durchläuft.

32. Verfahren nach Anspruch 31, wobei das Trennen der Komponenten der Probe das Anlegen eines Potentials an die Säule umfasst, so dass die Komponenten der Probe durch elektrophoretische Mobilität getrennt werden.

Revendications

1. Colonne de séparation (10 ; 100, 102) comprenant :

un substrat (11) ;
un axe longitudinal (16, 110) ;
une pluralité de structures de support monolithiques contiguës (2 ; 14; 30; 34; 38; 42; 46; 50, 50'; 64; 66; 68; 70; 84; 86; 94; 97) formées sur ledit substrat (11) et disposées en deux dimensions, afin de définir une pluralité de canaux interconnectés (6; 12; 31;32;35;39;43;47;49;60; 62;75;77;90;104,108,109) ; et
une entrée (96) et/ou une sortie (98) comprenant un réseau de canaux (99 ;118) qui divise séquentiellement un canal unique (112 ;114) en de multiples canaux (101 ;116) par X^n où « X » est le nombre de canaux dans lesquels un canal simple se divise et « n » est le nombre de fois où les canaux se divisent le long de l'axe longitudinal (16;110), dans lequel le réseau de canaux (99 ;118) de l'entrée/sortie (96 ;98) est défini par des structures monolithiques formées sur ledit substrat (11) et est en communication de fluide avec ladite pluralité de canaux interconnectés (6;12;31;32;35;39;43;47; 49;60;62;75;77; 90;104,108,109).

2. Colonne de séparation selon la revendication 1, dans laquelle chaque structure de support (14) de la pluralité de structures de support monolithiques contiguës (14) a une première extrémité (7) et une seconde extrémité (9) et les structures de support monolithiques contiguës (14) sont reliées les unes aux autres à la première extrémité (7) de chaque structure de support monolithique contiguë (14).

3. Colonne de séparation selon la revendication 2, comprenant en outre une plaque de couverture (13) reliée à chacune de la pluralité des structures de support monolithique contiguës (14) à la seconde extrémité (9) de chaque structure de support monolithique (14).

4. Colonne de séparation selon la revendication 2, dans laquelle la pluralité de structures de support monolithiques contiguës (2,14) comprend un premier et un second groupe de structures de support monolithiques contiguës, le second groupe étant une image en miroir du premier groupe, disposé au-dessus du premier groupe, de sorte que la seconde extrémité (9) de chaque structure de support monolithique (14) du premier groupe soit en communication avec la seconde extrémité (4) d'une structure de support monolithique correspondante (2) du second groupe, en fermant ainsi la colonne de séparation.

5. Colonne de séparation selon l'une quelconque des revendications 1 à 4, dans laquelle la pluralité des structures de support monolithique contiguës (2;14) présente des surfaces (1;15) capables d'interagir avec des analytes passant à travers la pluralité de canaux interconnectés (6;12) pour provoquer une séparation des analytes.

6. Colonne de séparation selon la revendication 5,

dans laquelle les surfaces (1;15) des structures de support monolithiques (2;14) sont revêtues d'un analyte de liaison spécifique.

7. Colonne de séparation selon l'une quelconque des revendications 1 à 6, dans laquelle la pluralité des canaux interconnectés (6;12;31;32;35;39;43;47; 49; 60;62;75;77;90;104,108,109) est sensiblement uniforme en forme et en taille.

8. Colonne de séparation selon l'une quelconque des revendications 1 à 7, dans laquelle la pluralité de canaux interconnectés est sensiblement constituée de canaux rectangulaires.

9. Colonne de séparation selon l'une quelconque des revendications 1 à 8, dans laquelle chacune de la pluralité des structures de support monolithiques contiguës (2;14; 38;42; 50;50';64; 70; 84; 94;97) a une coupe transversale tétragonale, dans laquelle la coupe transversale est un plan parallèle à un plan d'un substrat (11) supportant les structures de support.

10. Colonne de séparation selon l'une quelconque des revendications 1 à 8, dans laquelle chacune de la pluralité de structures de support monolithiques contiguës (30;34;66;68;86) a une coupe transversale hexagonale, dans laquelle la coupe transversale est un plan parallèle à un plan d'un substrat (11) supportant les structures de support.

11. Colonne de séparation selon la revendication 8 ou 9, dans laquelle un rapport d'aspect d'un canal entre deux structures de support adjacentes est dans la gamme d'environ 5 à environ 100, dans laquelle le rapport d'aspect est le rapport entre une profondeur et une largeur d'un canal, où la profondeur est une dimension perpendiculaire à un plan du substrat (11) supportant les structures de support et la largeur est une dimension perpendiculaire à un sens de flux dans le canal et parallèle au plan du substrat (11).

12. Colonne de séparation selon l'une quelconque des · revendications 1 à 11, dans laquelle le couplage intercanal est réalisé par la pluralité de canaux interconnectés définis par la pluralité de structures de support monolithiques contiguës, chaque structure monolithique contiguë ayant une longueur qui est sensiblement égale à une largeur, où la longueur est une dimension parallèle à et la largeur est une dimension perpendiculaire à un axe longitudinal de la colonne de séparation et parallèle à un plan du substrat.

13. Colonne de séparation selon l'une quelconque des revendications 1 à 12, dans laquelle le couplage in-

tercanal est réalisé par une configuration en forme de X ou Y des canaux d'intersection.

14. Colonne de séparation selon l'une quelconque des revendications 1 à 13, dans laquelle un courant électrophorétique est adapté pour être appliqué à la colonne et la chaleur créée par le courant est dissipée, par le biais de la pluralité de structures de support monolithiques.

15. Colonne de séparation selon l'une quelconque des revendications 1 à 14, dans laquelle chacune des structures de support monolithiques est dimensionnée pour optimiser la dissipation thermique.

16. Colonne de séparation selon l'une quelconque des revendications 1 à 14, dans laquelle l'efficacité de la séparation par longueur unitaire de la colonne de séparation est fournie par la pluralité de structures de support monolithiques contiguës, une zone en coupe transversale de chaque structure de support monolithique de la pluralité des structures de support contiguës mesurée parallèlement à un substrat supportant les structures de support ayant une longueur qui est sensiblement plus courte qu'une largeur, dans laquelle la longueur est parallèle à un axe longitudinal de la colonne de séparation et la largeur est sensiblement perpendiculaire à l'axe longitudinal de la colonne de séparation et parallèle à un plan du substrat.

17. Colonne de séparation selon l'une quelconque des revendications 1 à 16, dans laquelle les parois adjacentes des canaux (88) de la colonne de séparation sont sensiblement parallèles aux parois afin de sensiblement éliminer un effet de mur.

18. Colonne de séparation selon l'une quelconque des revendications 1 à 16, dans laquelle les angles des parois adjacentes des canaux (92) des colonnes de séparation (94) sont arrondis pour éliminer tout espace mort (80).

19. Colonne de séparation selon l'une quelconque des revendications 1 à 18, dans laquelle les zones en coupe transversale de tous les canaux (101;116) dans le réseau de canaux (99;118) sont sensiblement égales.

20. Colonne de séparation selon l'une quelconque des revendications 1 à 18, dans laquelle les zones en coupe transversale des canaux (101;116) dans le réseau de canaux (99;118) sont séquentiellement réduites quand le nombre de canaux (101;116) dans le réseau de canaux (99;118) augmente, en maintenant ainsi la zone en coupe transversale totale du réseau de canaux à travers tous les plans, perpendiculaires à l'axe longitudinal (16;110) de la

colonne de séparation, sensiblement constante.

21. Colonne de séparation selon l'une quelconque des revendications 1 à 20, dans laquelle la pluralité de canaux interconnectés (6;12;31;32;35; 39;43;47; 49;60;62;75;77;90;104,108,109) sont définis par la pluralité de structures de support monolithiques contiguës (2 ;14;30;34;38;42;46;50,50';64;66;68; 70; 84;86;94;97), de sorte qu'ils se rencontrent séquentiellement et se divisent.

22. Procédé de fabrication de la colonne de séparation, selon l'une quelconque des revendications 1 à 21, ledit procédé comprenant :

   la fourniture d'un substrat (11) ;
   la formation de motifs sur le substrat (11) pour désigner les zones du substrat (11) à graver ;
   la gravure du substrat (11) pour créer la pluralité de structures de support monolithiques contiguës (2;14) définissant la pluralité de canaux interconnectés et les structures monolithiques créant le réseau de canaux de l'entrée (96) et/ ou de la sortie (98) ; et
   la fermeture de la colonne de séparation.

23. Procédé selon la revendication 22, dans lequel

   la fermeture de la colonne de séparation comprend la fixation d'une plaque de couverture (13) sur une surface de la pluralité des structures de support monolithiques contiguës (14).

24. Procédé selon la.revendication 22, dans lequel

   la fourniture du substrat comprend la fourniture d'un premier et d'un second substrat ;
   la gravure du substrat comprend la gravure du premier et du second substrat pour créer les structures de support monolithique contiguës (2,14) et l'entrée (96) et/ou la sortie (98) sur le premier et le second substrat qui sont des images en miroir ; et
   la fermeture de la colonne de séparation comprend la disposition du second substrat sur le premier substrat, de sorte que chaque structure de support monolithique (14) du premier substrat soit en communication avec une structure de support monolithique correspondante (2) du second substrat, en fermant ainsi la colonne de séparation.

25. Procédé selon la revendication 22, 23 ou 24, dans lequel la gravure du substrat comprend la gravure anisotrope, la gravure LIGA, la gravure ionique réactive, ou la gravure par faisceau d'électrons.

26. Appareil de séparation comprenant :

une colonne de séparation selon l'une quelconque des revendications 1 à 21 ;
une pluralité de réservoirs en communication avec la colonne de séparation ; et
un réservoir d'échantillons en communication avec la colonne de séparation.

27. Appareil de séparation selon la revendication 26, comprenant en outre une pompe située de façon à pomper une phase mobile d'un réservoir à travers la colonne de séparation.

28. Appareil de séparation selon la revendication 26, comprenant en outre un appareil d'électrophorèse en communication électrique avec la colonne de séparation, l'appareil d'électrophorèse appliquant un potentiel à travers la colonne de séparation pour séparer les composants d'un échantillon passant à travers la colonne de séparation.

29. Appareil de séparation selon la revendication 26, comprenant en outre un détecteur en communication avec la colonne de séparation.

30. Appareil de séparation selon la revendication 29, dans lequel le détecteur est un spectromètre de masse.

31. Procédé de séparation de composants d'un échantillon, le procédé comprenant :

   l'introduction d'une solution d'un milieu dans l'entrée de la colonne de séparation, selon l'une quelconque des revendications 1 à 21 ;
   l'introduction d'un échantillon dans l'entrée de la colonne de séparation ; et
   la séparation des composants de l'échantillon quand l'échantillon passe à travers la colonne de séparation.

32. Procédé selon la revendication 31, dans lequel la séparation des composants de l'échantillon comprend l'application d'un potentiel à la colonne, de sorte que les composants de l'échantillon soient séparés par mobilité électrophorétique.
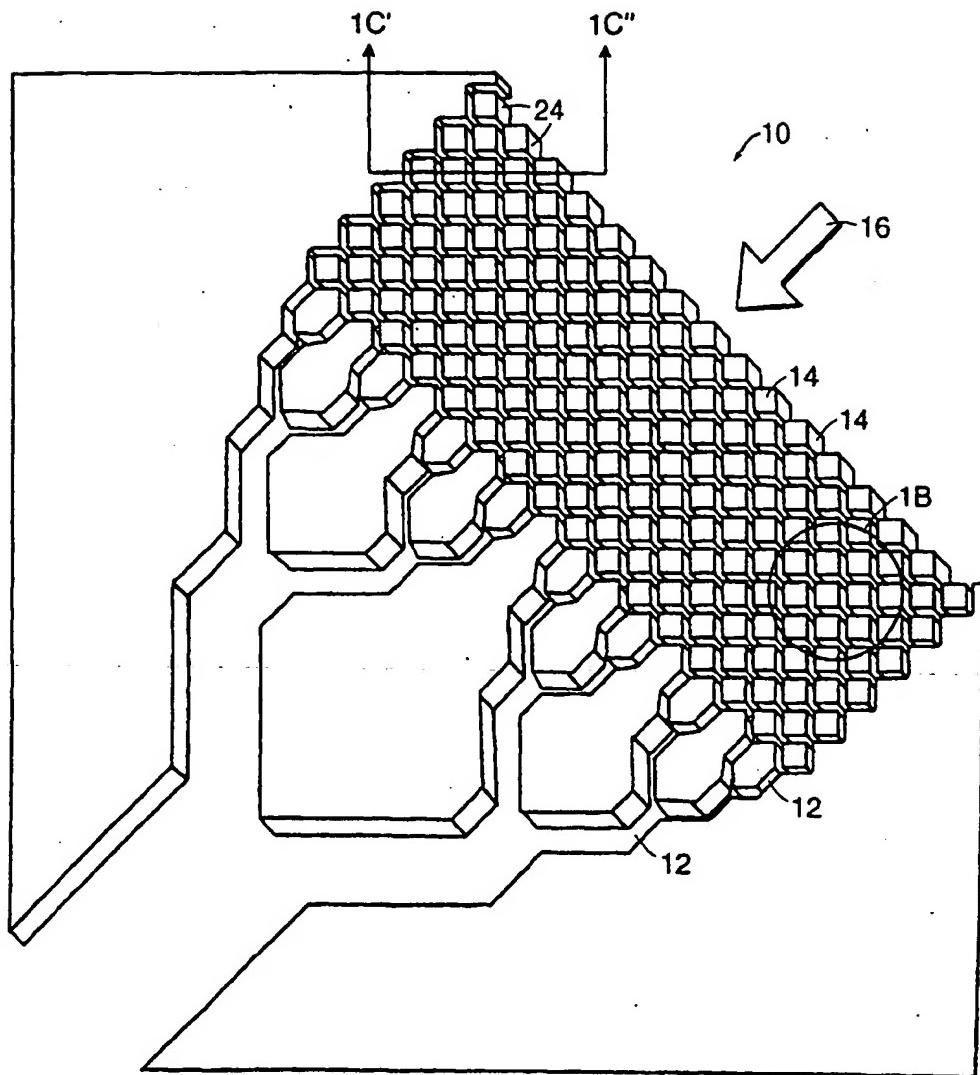
FIG. 1A

FIG. 1B

FIG. 1D

FIG. 1C

FIG. 2E
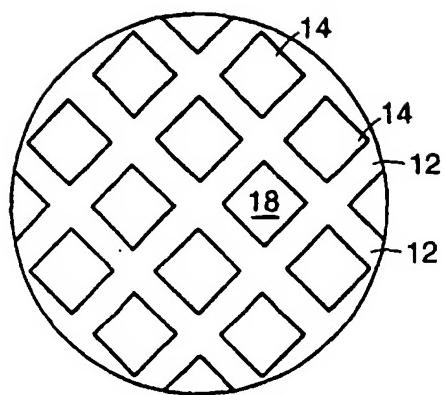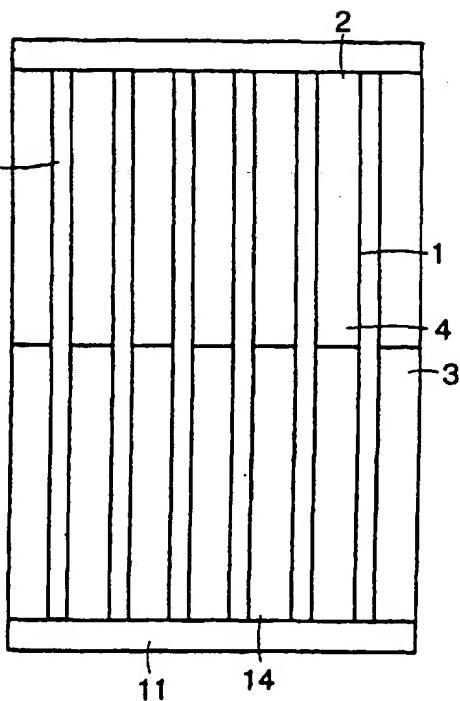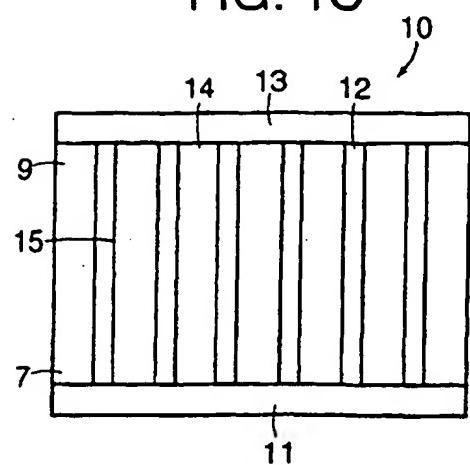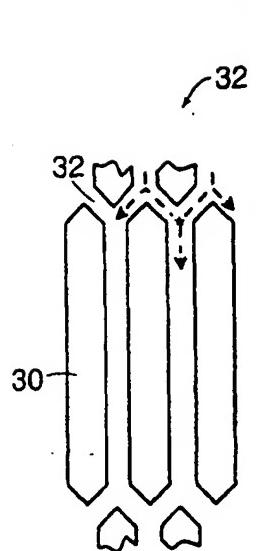
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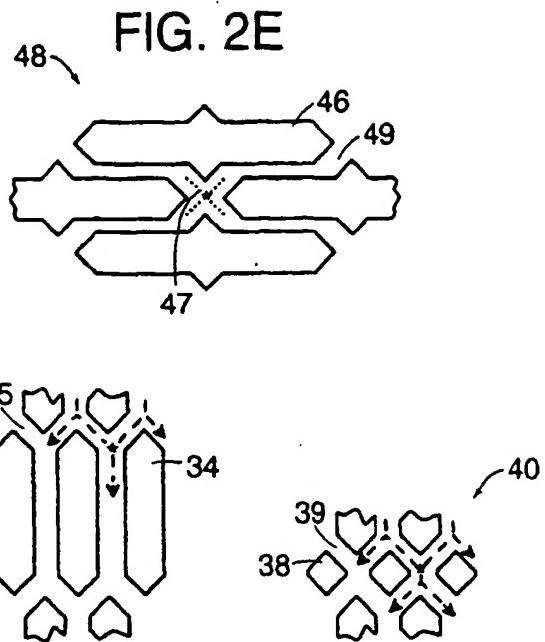
FIG. 2B

FIG. 2C
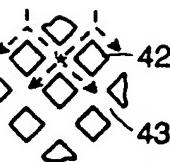
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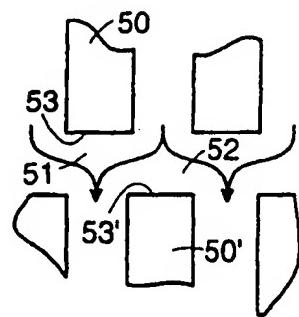
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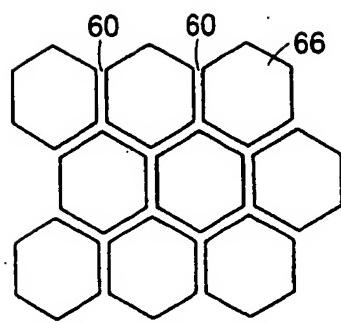
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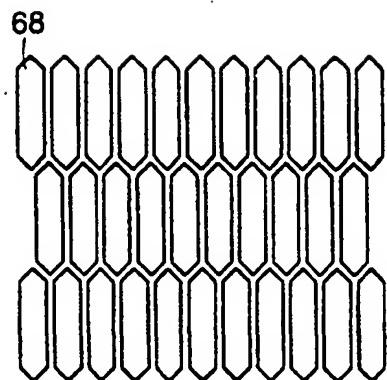
FIG. 3B
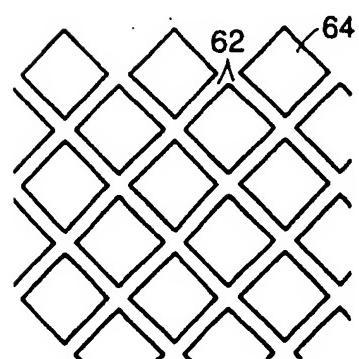


FIG. 3C
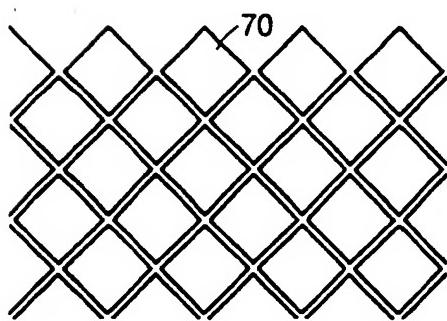
FIG. 3D
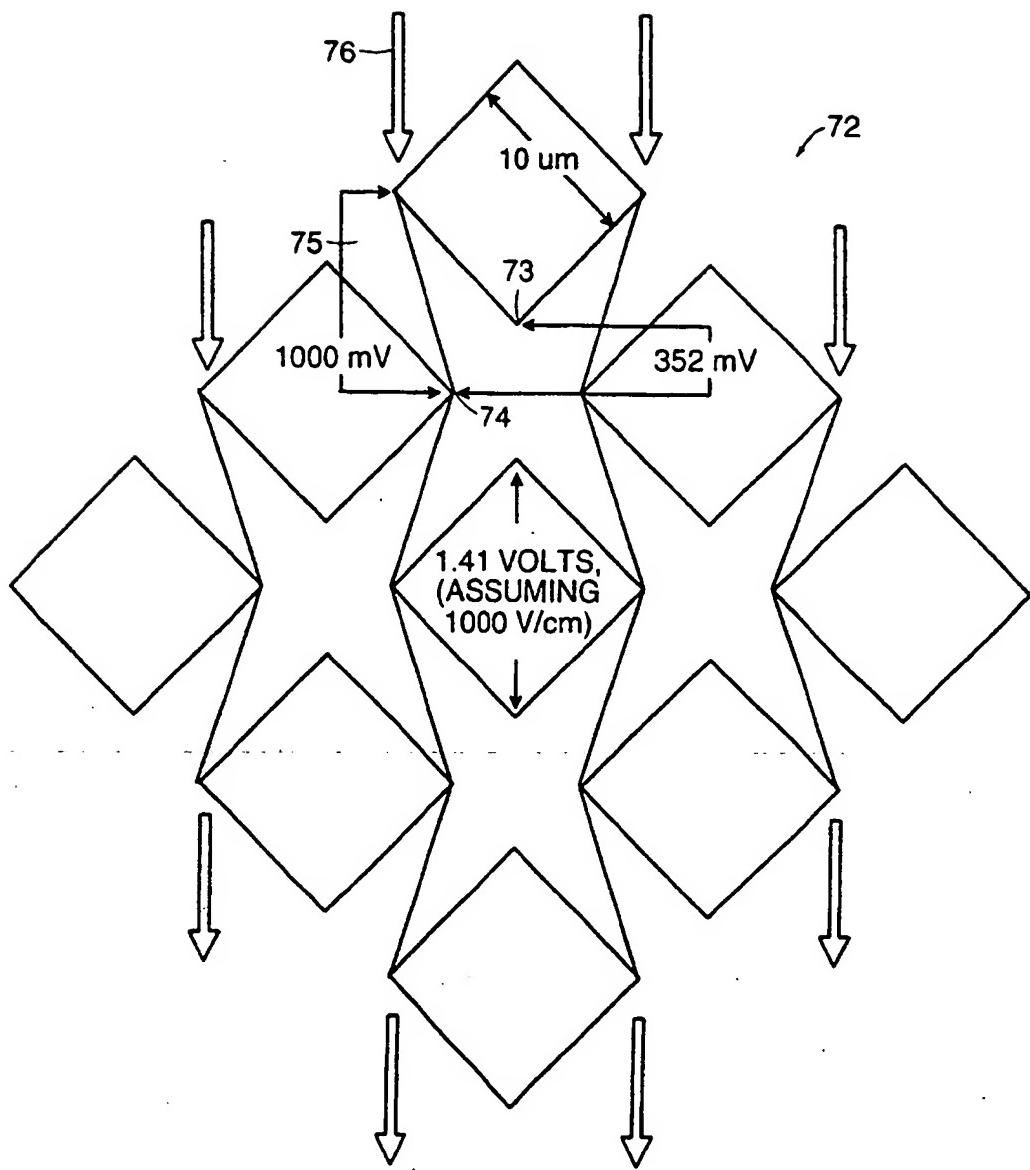
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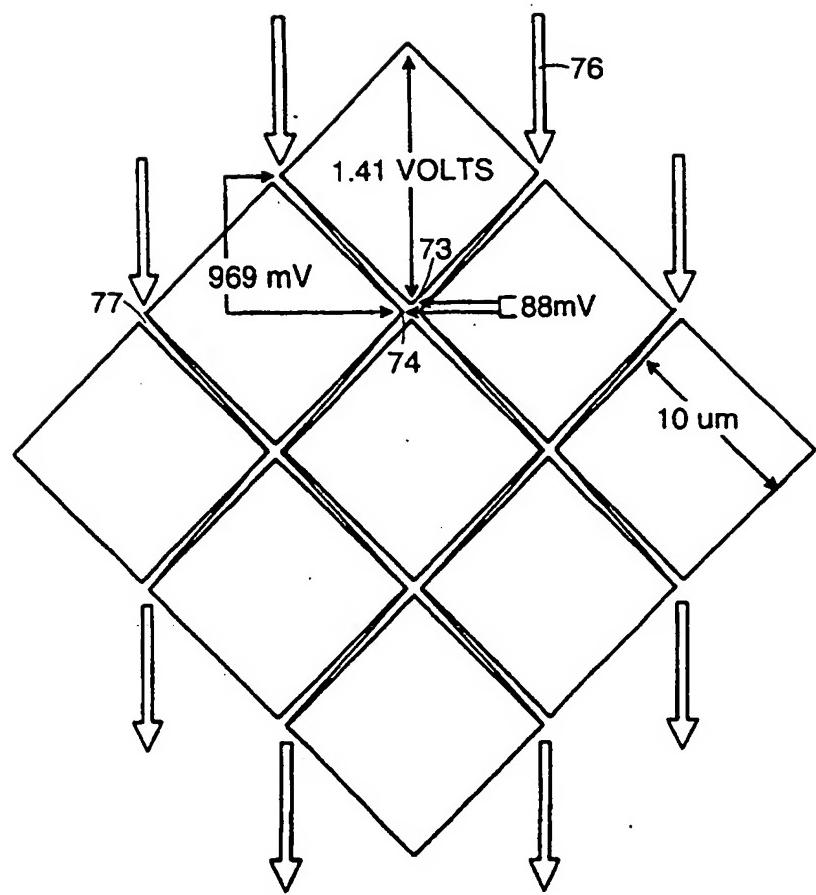
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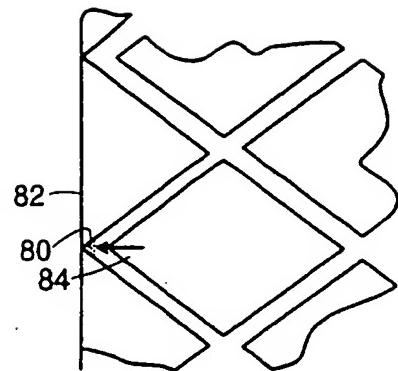
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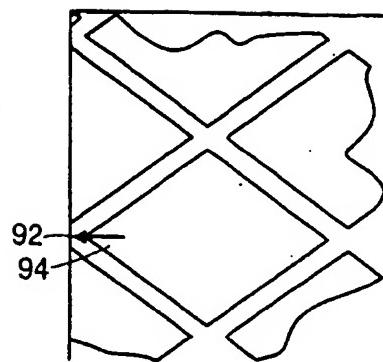
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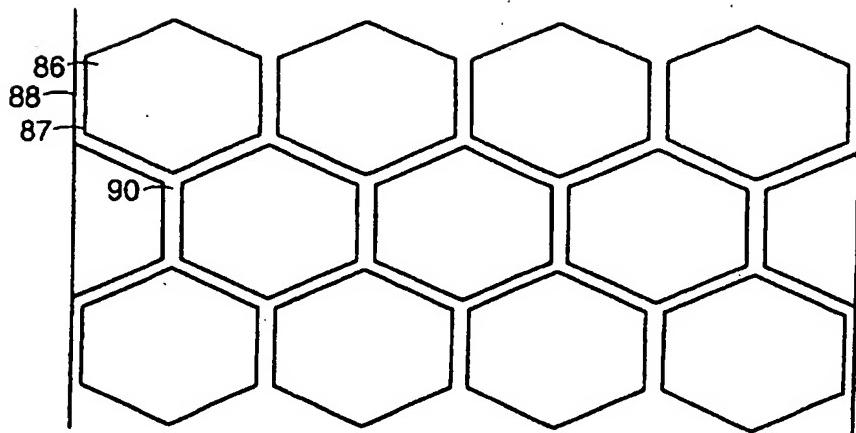
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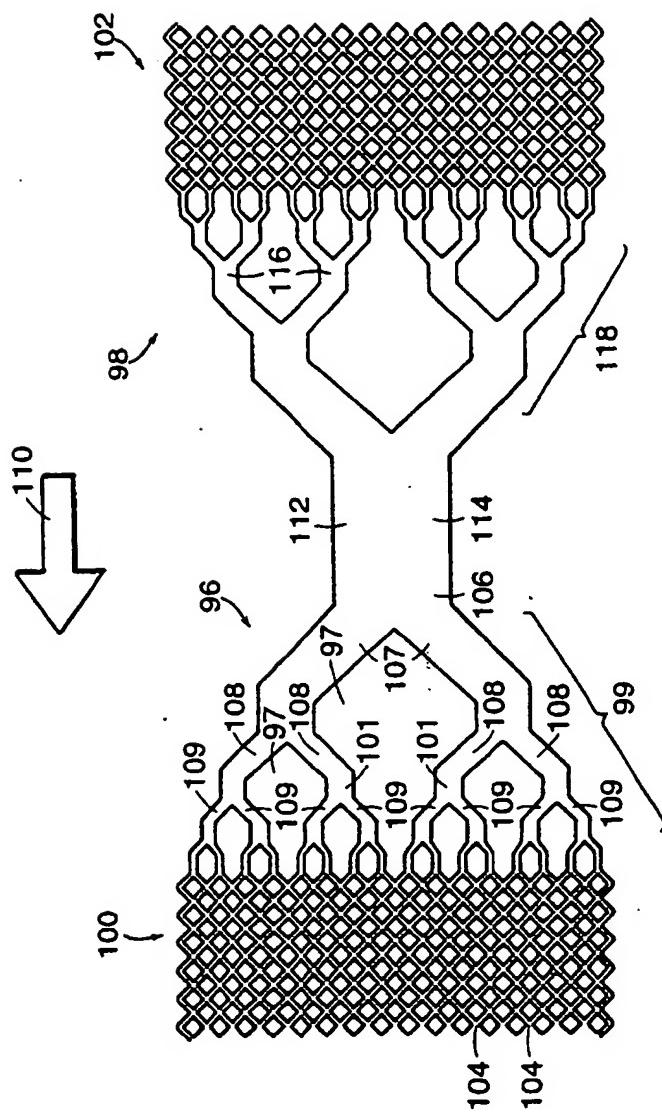
FIG. 6

FIG. 7

SEPARATION OF RHODAMINE & FLUORESCEIN
4.5 cm EFFECTIVE SEPARATION LENGTH
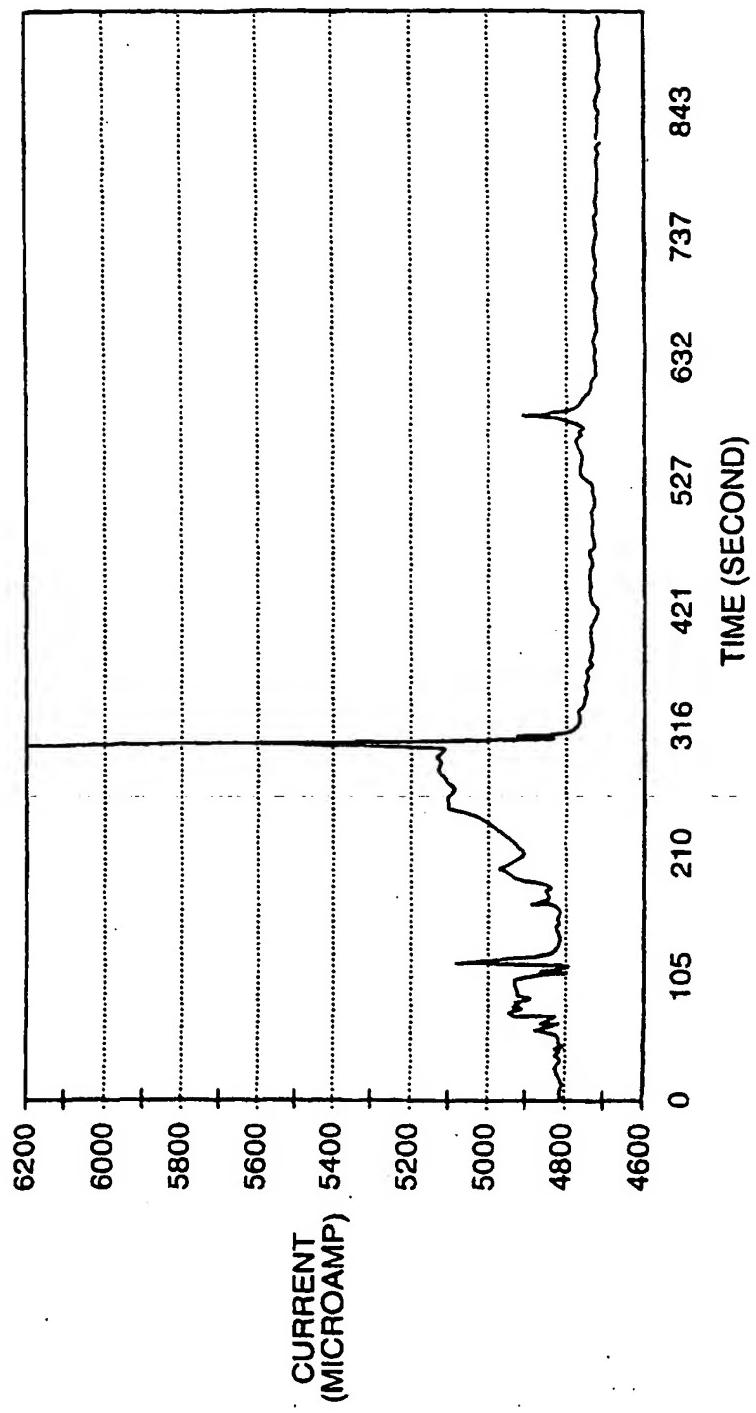
FIG. 8

SEPARATION OF PEPTIDES FROM HGH
4.5 cm EFFECTIVE SEPARATION LENGTH

FLUORESCENCE
INTENSITY
(NANOAMP)

TIME (SECOND)